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Research Article

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In Vivo Inhibition of Glucagon Secretion by Paracrine Beta Cell Activity in Man

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ABSTRACT The close anatomical relationships between pancreatic alpha and beta cells makes possible their interaction at a local (paracrine) level. To demonstrate this *in vivo*, we have compared the acute glucagon response to intravenous arginine in the basal state and after beta cell suppression by infusions of insulin. The plasma glucose concentration was maintained by the glucose clamp technique. In six normal weight nondiabetics, infusion of insulin at 0.2 mU/kg per min (rate 1) raised the mean \pm SEM plasma insulin levels from 10 ± 3 to 32 ± 4 mU/liter and at 1 mU/kg per min (rate 2) raised plasma insulin to 84 ± 8 mU/liter. This resulted in beta cell suppression, as shown by a diminution in the acute insulin response (incremental area under the insulin response curve, 0–10 min): basal = 283 ± 61 , 199 ± 66 (rate 1) and 143 ± 48 mU/liter per 10 min (rate 2) and a fall in prestimulus C-peptide from 1.05 ± 0.17 to 0.66 ± 0.15 and to 0.44 ± 0.15 nM/liter (all $P < 0.01$). This beta cell suppression was associated with increased glucagon responses to arginine: 573 ± 75 (basal), 829 ± 114 (rate 1), and 994 ± 136 ng/liter per 10 min (rate 2) and increased peak glucagon responses 181 ± 11 (basal), 214 ± 16 (rate 1), and 259 ± 29 ng/liter (rate 2) (all $P < 0.01$). In all subjects, there was a proportional change between the rise in the acute glucagon response to arginine and the fall in the prearginine C-peptide level. To demonstrate that augmented glucagon response was due to beta cell suppression, and not to the metabolic effect of infused insulin, similar studies were performed in C-peptide-negative-diabetics. Their acute glucagon response to arginine was

inhibited by the insulin infusion: 701 ± 112 (basal), 427 ± 33 (rate 1), and 293 ± 67 ng/liter per 10 min (rate 2) as was their peak glucagon response: 268 ± 69 , 170 ± 36 , and 115 ± 33 ng/liter (all $P < 0.01$). Thus, hyperinsulinemia, within the physiological range achieved by insulin infusion, inhibits beta cell secretion which, via a paracrine mechanism, potentiates glucagon secretion.

INTRODUCTION

The regulation of pancreatic alpha cell secretion is probably mediated by complex interactions between multiple factors that include neural influences, circulating fuels, and gastrointestinal hormones. In addition, the close anatomical relationship between hormonally active cells in the islet of Langerhans makes it very likely that alpha cell secretion is influenced by the local concentration of other islet hormones (1). Such a local action has been termed "paracrine" in contrast to the endocrine action of pancreatic hormones, which is mediated through general circulation. The ability of insulin to inhibit glucagon secretion has previously been demonstrated *in vitro* in isolated islets, pancreatic pieces (2), or whole pancreas (3–5).

The administration of exogenous insulin *in vivo* causes many changes in circulating fuels (especially glucose) and hormones, in particular the suppression of endogenous insulin secretion (6–8). Any effect this fall in endogenous paracrine insulin secretion may have on glucagon secretion cannot be discerned because of simultaneous changes in circulating fuels and hormones that also affect glucagon release. The glucose clamp technique (9) enables the circulating level of insulin to be raised without changing plasma glucose concentration. Endogenous insulin secretion, as measured by C-peptide determinations, is inhibited (6–8) and thereby provides a model to investigate the paracrine effect of insulin on A cell secretion independent of any change in plasma glucose. We have, therefore, measured the acute glucagon responses to intravenous arginine in normal men, before and after insulin infusions, using the glucose clamp technique.

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TABLE I
Mean (\pm SEM) Glucose, Insulin, Glucagon, and C Peptide Levels before Arginine Administration and the Hormonal Response to Arginine, in Nondiabetics and Diabetics, in the Basal State and during Insulin Infusions

		Basal	Insulin infusion	
			Rate I	Rate II
Nondiabetics				
Prearginine	Plasma glucose, mg/dl	86 \pm 6	86 \pm 7	86 \pm 7
	Plasma IRI, mU/liter	10 \pm 3	32 \pm 4	84 \pm 8
	Plasma IRG, ng/liter	78 \pm 15	56 \pm 10	64 \pm 10
	Plasma IR* C-peptide, nmol/liter	1.05 \pm 0.17	0.66 \pm 0.15	0.44 \pm 0.15
Postarginine	AIR \dagger , mU/liter per 10 min	283 \pm 61	199 \pm 66	143 \pm 48
	AGR, ng/liter per 10 min	573 \pm 75	829 \pm 114	994 \pm 136
	Peak IRG, ng/liter	181 \pm 11	214 \pm 16	259 \pm 29
Diabetics				
Prearginine	Plasma glucose, mg/dl	190 \pm 45	191 \pm 44	190 \pm 45
	Plasma IRG, ng/liter	114 \pm 33	87 \pm 35	82 \pm 11
Postarginine	ARG, ng/liter per 10 min	701 \pm 112	427 \pm 33	293 \pm 67
	Peak IRG, ng/liter	268 \pm 69	170 \pm 36	155 \pm 33

* IR, immunoreactive.

\dagger AIR, incremental area under the insulin response curve; 0–10 min.

METHODS

Six young male, normal weight nondiabetic subjects with no family history of diabetes in first degree relatives, and six age-, sex-, weight-matched insulin-dependent diabetics were studied. The diabetics had had the onset of their disease in early childhood and required insulin therapy for 10 yr or more. Following an overnight fast and subject to informed consent, intravenous cannulae were inserted into both antecubital veins and into a vein on the back of the hand. The hand was placed in a thermostatically controlled "hot hand box" for the collection of "arterialized" venous blood (10). After a 45-min basal period, during which blood was drawn at 15 min intervals, a 5 g i.v. bolus of arginine was administered over 30 s. Blood samples were withdrawn at 2, 3, 4, 5, 6, 8, 10, 15, 20, and 30 min following the arginine injection. An infusion of high purity porcine insulin (Eli Lilly and Co., Indianapolis, Ind.) in saline was started using a 50-ml syringe pump and 10 ml of the subject's own blood (to minimize the surface absorption of insulin [11]). Insulin was infused at 0.2 mU/kg per min for 90 min (rate I), blood being drawn at 5-min intervals for 60 min, then another bolus of arginine was given with blood sampling as above for 30 min. The insulin infusion rate was then changed to 1 mU/kg per min (rate II), and the procedure as for rate I followed. Half the subjects in each group had insulin delivered at rate II before rate I. During the insulin infusions, blood glucose was maintained at basal levels by a 5 or 10% dextrose infusion via the glucose clamp technique, and arterialized blood glucose concentrations were determined every 5 min. Blood samples were collected into chilled (4°C) tubes with samples for glucagon being collected into benzamidine, centrifuged immediately, and frozen at -20°C until analysis.

Plasma immunoreactive insulin (IRI)¹ and glucagon (IRG)

¹ Abbreviations used in this paper: AGR, acute glucagon response; AIR, acute insulin response; IRG, immunoreactive glucagon; IRI, immunoreactive insulin.

were measured by radioimmunoassay using double antibody (12) and polyethylene glycol (13) separation, respectively. Serum C-peptide was measured after polyethylene glycol extraction using dextran-charcoal separation and antibody M1230 (Novo) (14). Blood glucose was measured on whole blood using a Yellow Springs Analyzer, results being available 40 s after sample collection. The hematocrit was used to correct whole blood to plasma glucose values. In some subjects the serum potassium was also measured. The acute glucagon response (AGR) to arginine was calculated as the incremental area under the IRG response curve from 0–10 min following the arginine injection. Similarly, the acute insulin response (AIR) was calculated in the nondiabetics. Results are shown as mean \pm SEM and the statistical significant differences between groups were tested by Wilcoxon rank.

RESULTS

Blood glucose levels were maintained constant at basal values (Table I) in all subjects during the insulin infusion (CV < 3%). Suppression of endogenous insulin secretion in the nondiabetic subjects was documented by the progressive fall in prestimulus C-peptide levels from a mean basal level of 1.05 \pm 0.17 nmol/liter to 0.66 \pm 0.15 at insulin rate I, and to 0.44 \pm 0.15 at insulin rate II. In addition, the AIR to arginine fell in all subjects, being 283 \pm 61 mU/liter per 10 min in the basal state, and 199 \pm 66 and 143 \pm 48 mU/liter per 10 min at insulin rates I and II, respectively (P < 0.01 for all comparisons) (Table I and Fig. 1).

In all the nondiabetic subjects, the AGR to arginine was greater at insulin infusion rate I (829 \pm 114 ng/liter per 10 min) than at basal level (573 \pm 75 ng/liter per 10

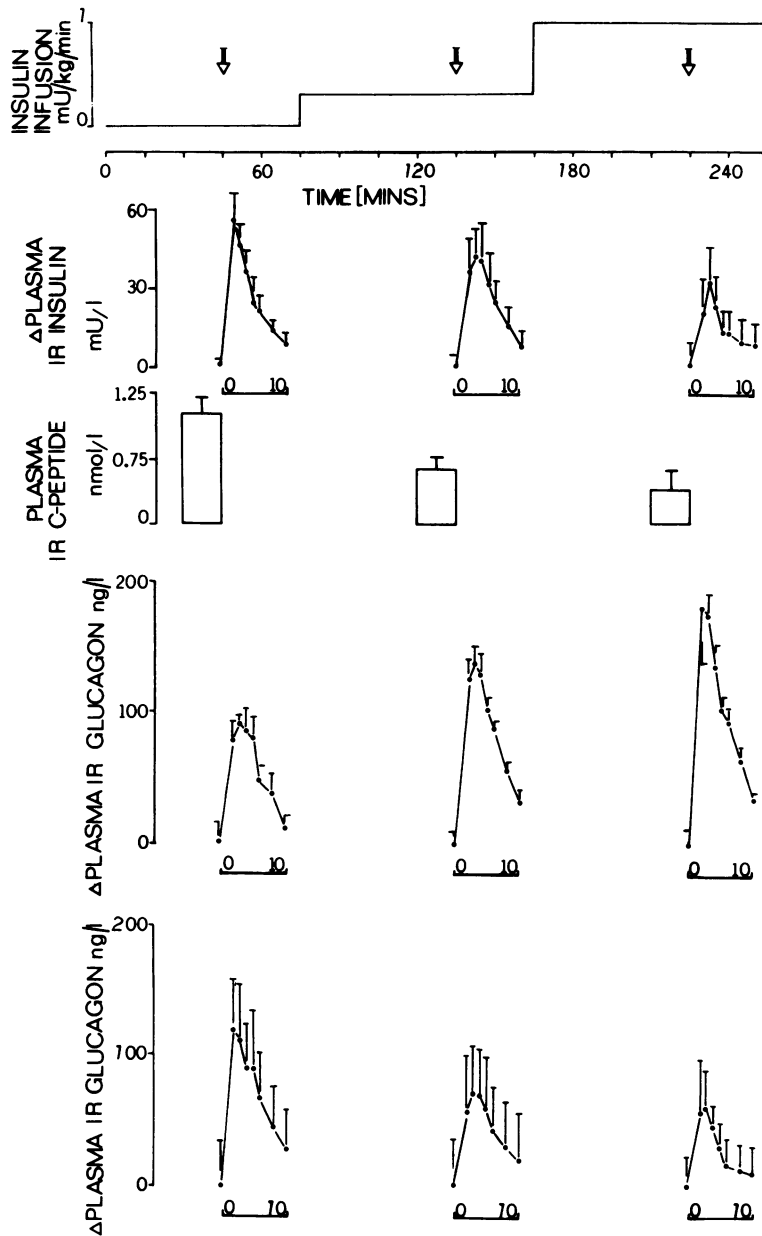


FIGURE 1 Insulin infusion rates (mU/kg per min), plasma glucose concentrations (mg/dl), plasma IRI (mU/liter), immunoreactive (IR)C-peptide levels prearginine, (nmol/liter), and IRG levels in six nondiabetics and six diabetics. Data are presented as $\bar{X} \pm \text{SEM}$; arg ↓ indicates time of arginine injection; l, liter.

min) and a further rise occurred during insulin infusion rate II (994 ± 136 ng/liter per 10 min) ($P < 0.01$ for all comparisons, see Table I and Fig. 1). In all subjects there was a strong relationship between prestimulus C-peptide levels and the AGR although the slope of these lines was variable (Fig. 2). The peak IRG response postarginine, irrespective of time, also increased progressively: basal = 181 ± 11 , rate I = 214 ± 16 , rate II = 259 ± 29 ng/liter ($P < 0.01$) (Table I).

None of the diabetics had detectable circulating C-peptide levels. Their basal AGR was 701 ± 112 ng/liter per 10 min and the AGR fell to 427 ± 33 ng/liter per 10 min when insulin was infused at rate I and fell further to 293 ± 67 ng/liter per 10 min at insulin rate II ($P < 0.01$). Similarly, the peak IRG level postarginine, irrespective of time, also decreased in every diabetic during the infusion of insulin (Fig. 1 and Table I). Three of the diabetics studied were normoglycemic

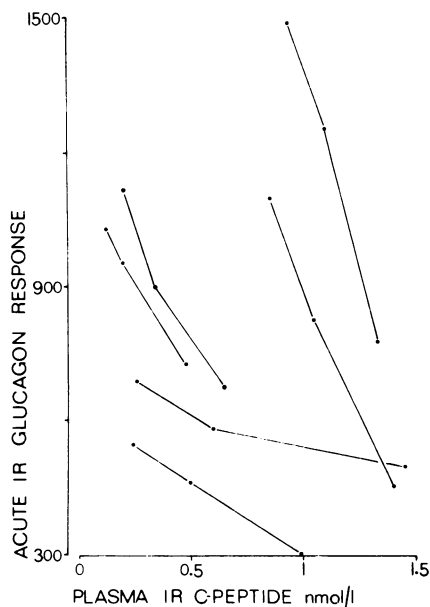


FIGURE 2 AGR to arginine (ng/liter per 10 min) for six nondiabetics related to their prearginine immunoreactive (IR) C-peptide levels (nmol/liter). l, liter.

at the time of the study. We have also studied six additional diabetics (two of whom were also normoglycemic) in the basal state and after insulin infusion (rate II). The basal AGR (596 ± 41 ng/liter per 10 min) and the AGR at insulin infusion rate II (328 ± 70 mg/liter per 10 min) in these five normoglycemic (mean plasma glucose = 82 ± 10 mg/dl) diabetics were similar to the responses seen in the diabetics as a group. Also, there was no correlation between the AGR in the basal state and the fall in the AGR as a result of the insulin infusion and the plasma glucose level.

The prestimulus IRG levels fell in both nondiabetics and diabetics from basal values of 78 ± 15 ng/liter and 114 ± 33 ng/liter, respectively, to 56 ± 10 ng/liter and 87 ± 35 ng/liter during insulin infusion rate I, and to 64 ± 10 ng/liter and 82 ± 11 ng/liter during insulin infusion rate II. No consistent change in serum potassium was noted, the greatest change being 0.2 mmol/liter.

DISCUSSION

This study has demonstrated enhancement of the alpha cell response to arginine during suppression of beta cell secretion in nondiabetics. This suggests that the beta cell, functioning in a paracrine fashion, inhibits alpha cell secretion in vivo, similar to the inhibition of glucagon secretion by insulin in vitro (2-5). This has not been previously demonstrated in man.

Suppression of endogenous insulin in the nondiabetics is shown by the progressive fall in prestimulus C-peptide levels and by the fall in AIR to arginine

in all subjects. Thus, when the plasma glucose level remains constant, endogenous insulin secretion can be suppressed by insulin infusion rates that give circulating insulin levels within the physiological range. In previous studies, supraphysiological (7) and physiological (6) serum insulin levels have been used to suppress endogenous insulin secretion (as shown by C-peptide levels). In the isolated pancreas, insulin itself (8) will suppress beta cell insulin secretion, however, in this study, it is not known whether the exogenous insulin suppressed beta cell function directly, or via a change in one of many circulating fuels (other than glucose), hormones, or by a combination thereof. In nondiabetics, the overall effect on the pancreatic alpha cell was a progressively greater AGR to arginine during the insulin infusions, probably due to a lesser A cell suppression by paracrine beta cell activity. In diabetics, who lack any detectable beta cell secretory capacity, this response was not observed; in fact, the opposite, inhibition of the AGR to arginine, was found.

The changes in AGR are not merely secondary to changes in prestimulus IRG levels because peak IRG after the arginine pulse, irrespective of time, rose progressively in all nondiabetics and fell progressively in all diabetics. The incremental area is calculated from the lowered basal level. However, the alpha cells could have been affected by changes in circulating free fatty acids, ketone bodies, or amino acids brought about by the insulin infusions. Since the circulating fuels in diabetics and nondiabetics would be expected to show similar qualitative changes during the insulin infusions, whereas the glucagon responses we observed were in opposite directions, it is unlikely that the rises in AGR in nondiabetics during the insulin infusion are a result of some change in levels of circulating fuels. The similarity of AGR seen when normoglycemic and hyperglycemic diabetics were compared makes it unlikely that the differences observed between diabetics and nondiabetics are secondary to differences in circulating fuels. This is further supported by the absence of any relationship between the plasma glucose level and basal AGR or the degree of fall in the AGR brought about by insulin infusion. It is more likely that the difference between nondiabetics and diabetics is due primarily to differences in beta cell paracrine inhibition of alpha cells.

The prestimulus IRG levels fell in both diabetics and nondiabetics. This is presumably partly in response to changes in circulating fuels other than glucose brought about by the insulin infusion, but is complicated by the lack of knowledge of the source of IRG measured in the basal state. In contrast, the IRG seen after arginine injection appears to be 3,500 dalton glucagon of pancreatic origin. In support of this possibility is the observation that in pancreatectomized

man basal IRG is often normal but the response to arginine is minimal (15). It is possible that basal glucagon is largely nonpancreatic in origin, therefore not under paracrine insulin influence, and is inhibited by exogenous insulin (16) similar to pancreatic alpha cells in insulin-dependent diabetics who lack paracrine beta cells. Thus, studies in which insulin, with or without glucose, has been infused into diabetics and nondiabetics followed by changes in basal IRG, are not comparable with the arginine stimulated IRG data in the present study (5, 17, 18).

The importance of paracrine insulin in inhibiting alpha cell secretion might provide an explanation for the observations that during insulin induced hypoglycemia, insulin dependent diabetics have a markedly deficient or absent IRG response compared to nondiabetics (19, 20). The IRG response to hypoglycemia would be comparable to the stimulated (by arginine) IRG response presented here. Hypoglycemia would suppress paracrine insulin in nondiabetics resulting in decreased inhibition of the alpha cell. If this was an important factor in IRG response to hypoglycemia, insulin dependent diabetics who lack such a mechanism would be expected to have an impaired IRG response. Also, the inconsistent IRG responses of insulin dependent diabetics (without hypoglycemia) to insulin may be related to the varying degrees of beta cell destruction in these diabetics (21).

Previous morphologic and in vitro observations have suggested that beta cells affect alpha cell secretion by a paracrine mechanism (1-5). Our data extend these observations to man and suggest that the disturbed paracrine relationship of alpha and beta cells in diabetes may explain, in part, the abnormal glucagon dynamics observed in diabetes.

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